Development of a Mathematical Model for a proposed 7-ketocholesterol degradation pathway in *Rhodococcus jostii* using Michaelis-Menten Enzyme Kinetics

Overview

During 2014, iGEM ITESM CEM Team worked on the development of a metabolic pathway for 7-ketocholesterol degradation by enzyme therapy of human macrophages, using two hypothetic enzymes of the microorganism *Rhodococcus jostii*, active when the microbe grows using 7-ketocholesterol as a sole source of carbon. These enzymes (oxoacyl reductase and 7-dehydratase) were first described as potentially being used to catalyse these reactions by Mathieu (1); and were cloned and purified by iGEM ITESM CEM Team using *E. coli* and a diverse array of expression vectors.

However, in order to properly asses and predict the behaviour of both proteins in the cytosol of human cells, it is first necessary to numerically model their interaction and catalysis over their substrates. In order to do so, the proposed pathway must firstly be analysed.

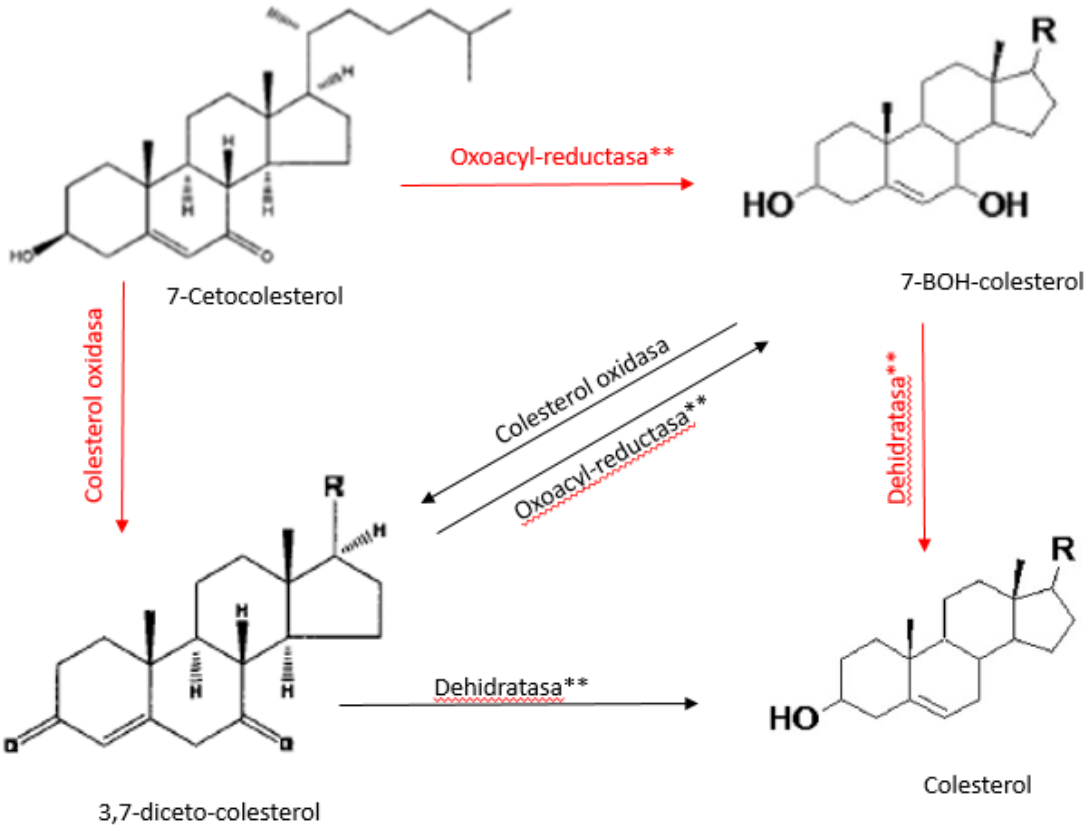

Figure 1.- Theoretical metabolic pathway for 7-ketocholesterol degradation in *Rhodococcus jostii*. Red arrows indicated the most intuitive order of reactions.

Figure 1 shows the general array of chemical reactions in the pathway, were red arrows indicate the direction proposed by iGEM ITESM CEM Team. Even though reactions can occur in almost any order, the most intuitive arrangement is that in which 7-ketocholesterol is converted to 7-βOH-cholesterol, which is finally transformed to regular cholesterol; these two reactions are supposed to be catalysed by oxoacyl reductase and 7-dehydratase respectively.

7-ketocholesterol is commonly present throughout the bloodstream in the form of esters (with a fatty acid chain attached to the 3rd position of the core rings) as a part of oxidized lipoproteins (usually oxLDL); 7-ketocholesterol is released from these particles by a transesterification reaction catalysed by cholesterol oxidase (the third enzyme cloned by the team). As for cholesterol, it is able to enter a wide variety of naturally occurring metabolic pathways such as hormone synthesis, bile salt production or, in a lesser extent, complete mineralization (CO_2 is formed). On the other hand, cholesterol is at the same time synthesized endogenously by human cells, and a small fraction of it comes from an animal product-containing diet.

Inside human macrophages (cells that are responsible for 7-ketocholesterol degradation throughout the development of atherosclerosis), this metabolic pathway does not exist. This is mainly due to the lack of specific enzymes that act upon oxidized derivatives of sterols, particularly 7-ketocholesterol. This is the main reason why using proteins derived from microbial genomes (in this study, *Rhodococcus jostii*) seems a reasonable approach in order to enhance 7-ketocholesterol metabolism, and minimize its accumulation on the inner walls of human arteries.

Since all the previously described chemical reactions are properly modelled using single-substrate enzyme kinetics, Michaelis-Menten equations were used. In order to simplify the proposed mathematical model, cholesterol metabolism was not included among the equations, and only the two enzymes directly involved in 7-ketone removal were modelled. This permits us to analyse the impact of variations in kinetic constants (Km and Rmax) over sterol metabolism, which lets us in turn asses the efficiency of a microbial pathway when compared to the endogenous degradation of 7-ketocholesterol in human cells.

Model Description

The general Michalies-Menten model was used, which was stated in a differential form, as the following:

$$R(S) = \frac{dS}{dt} = \frac{Rmax\ .\ S}{S + Km}$$

Where Km, as usually, represents the substrate concentration needed to reach one half of the maximum reaction rate, a measurement of enzyme's affinity for its substrate, and Rmax is the maximum reaction rate that can be achieved under the analysed pH, temperature, pressure and enzyme concentration conditions. In this case, three of these models were generated: each one describing the reaction rate of each component of the pathway (7-ketocholesterol, 7-βOH-cholesterol, and cholesterol).

In order to specifically focus on 7-keto removal, no 7-ketocholesterol synthesis or uptake was considered; and no cholesterol metabolism was included among the model. This means that an initial concentration of 7-ketocholesterol is gradually degraded until it reaches zero, and that cholesterol slowly builds up until it reaches a limit concentration. As for 7- βOH-cholesterol, its concentration varies according to the rate of reaction of both enzymes, firsts accumulating and then being degraded.

The following system of differential equations was generated, where each compound has a particular abbreviation (7KC for 7-ketocholesterol, 7βOHC for 7-βOH-Cholesterol, and Ch for Cholesterol:

$$\frac{d7KC}{dt} = 0 - \frac{Rmax1\ .\ 7KC}{Km1 + 7KC}$$

$$\frac{d7\beta\mathrm{OH}C}{dt} = \frac{Rmax1\ .\ 7KC}{Km1 + 7KC} - \frac{Rmax2\ .\ 7\beta\mathrm{OH}C}{Km2 + 7\beta\mathrm{OH}C}$$

$$\frac{dCh}{dt} = 0\ ;\ when \frac{d7\beta\mathrm{OHC}}{\mathrm{dt}} > 0$$

$$\frac{dCh}{dt} = \frac{Rmax2\ .\ 7\beta\mathrm{OH}C}{Km2 + 7\beta\mathrm{OH}C} - \frac{Rmax1\ .\ 7\mathrm{K}C}{Km1 + 7\mathrm{K}C} - 0\ ;\ when \frac{d7\beta\mathrm{OHC}}{\mathrm{dt}} \leq 0$$

This means that the concentration of 7-ketocholesterol is always described by the Michaelis-Menten reaction rate of the first enzyme, which tends to degrade it (a zero term stands for the non-existing 7-ketocholesterol synthesis); the concentration of 7- βOH-cholesterol is described by both the first and the second enzyme Micahelis-Menten kinetics, where the first one models its synthesis and the second one its degradation. Finally, cholesterol concentration is zero in the first stage of the reaction (when 7- βOH-cholesterol has not been metabolized yet), and is modelled by the second enzyme kinetics in the second reaction stage; here, it is generated at a reaction rate of the same magnitude and opposite sign of that of 7-βOH-Cholesterol degradation (a zero term stands for the non-existing cholesterol metabolism).

Because of the difficulty in solving this system of differential equations analytically, a numerical approach was used. In order to do so, the Runge-Kutta method of fourth order was used, which approximates the solution of a differential equation given 4 constants that are defined as shown in figure2 (2).

$$x_0, y_0 \rightarrow \begin{aligned} x_{i+1} &= x_i + h, \\ k_1 &= hf(x_i, y_i), \\ k_2 &= hf\left(x_i + \frac{h}{2}, y_i + \frac{k_1}{2}\right), \\ k_3 &= hf\left(x_i + \frac{h}{2}, y_i + \frac{k_2}{2}\right), \\ k_4 &= hf(x_i + h, y_i + k_3), \\ y_{i+1} &= y_i + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4) \end{aligned}$$

Figure 2.- Equations used for the iterative solution of differential equations using Runge-Kutta's fourth order method.

These iterative equations were programmed using Wolfram Mathematica; the results are presented in the following section.

Model Predictions

The three coupled Runge-Kutta numerical approximation methods were performed between times 0 and 0.01, with varying parameter values. In order to perform a comparative assessment between the efficiency of the endogenous degradation of 7-ketocholesterol, and our proposed synthetic pathway, some of the plots generated are included in figure 3. Because all of the enzymes cloned by iGEM ITESM CEM Team are supposed to be targeted to the lysosome via a signal peptide, they are assumed to work at low values of pH; this in turn promotes a high reaction rate for 7- βOH-Cholesterol degradation, because the hydroxyl group at the 7^{th} position of the core rings becomes a strong nucleophile when surrounded by an acidic environment, and because usually this step of the pathway must be fast enough so that this intermediate, very reactive with lipid bilayers, does not accumulate. This is the reason why both the maximum rate (Rmax2) and the Michaelis constant (Km2) of the second enzyme were fixed to be very high and very low, respectively. Based of similar enzymes' parameters reported in BRENDA, Rmax = 1100 and Km2 = 0.5 were used, this accounts for a fast reaction rate of reaction.

As for the first reaction, it is well known that the problem that ultimately enhances the development of atherosclerotic plaques is the inability of human cells to metabolize 7-ketocholesterol. This is due to a lack of a specific enzyme that performs this task; which ultimately causes this action to be performed by non-specific proteins which most probably have quite high Km values, which cause a really low affinity of the enzymes when acting upon this substrate. This is why figure 3 presents 5 plots with decreasing values of Michaelis constants for the first reaction (Km1). Here the maximum rate of reaction was fixed at a value slightly lower than that for the second one (fixed to 1000).

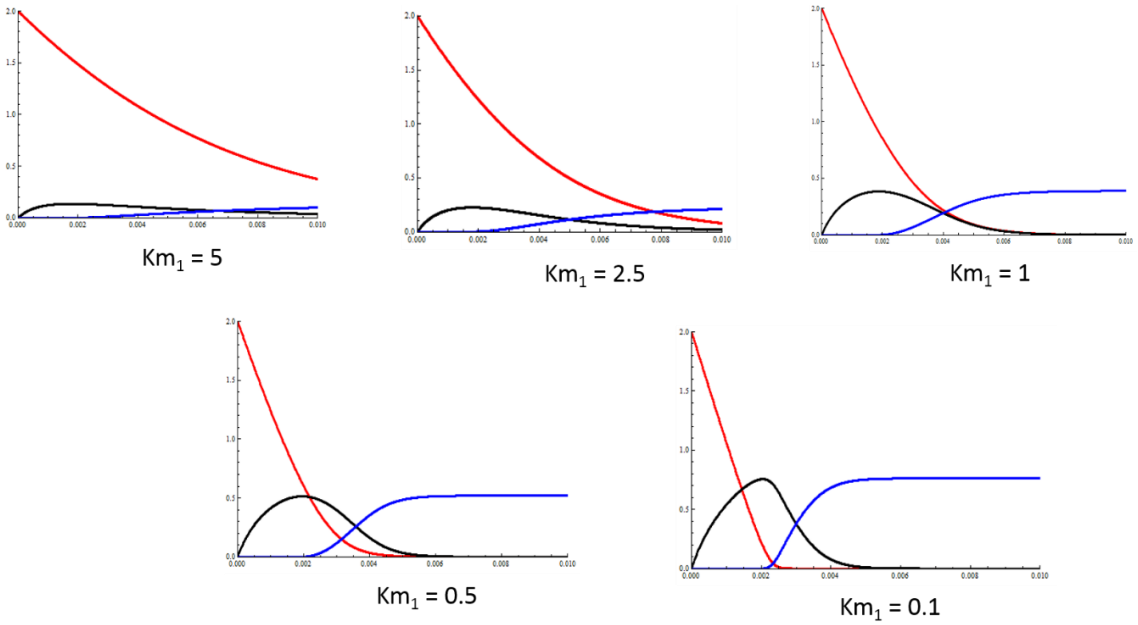

Figure 3.- Plots generated by the numerical solution of the differential model of enzyme kinetics using Runge-Kutta's fourth order method. Decreasing values for Michaelis constant for the first reaction are shown in each plot.

The first plot might accurately represent the situation inside human macrophages, where 7-ketocholesterol cannot be degraded at an appropriate rate, and gradually accumulates. If we further consider that this model does not include the endogenous synthesis and the uptake of 7-ketocholesterol, we can easily see why this molecule's concentration rapidly builds up in the bloodstream. However, as Km values for this reaction diminish, the plots tend to represent more efficient metabolic pathways: we expect our synthetic route, based of *Rhodococcus jostii*'s sterol metabolism, to be modelled by one of the last two plots in the figure; here, 7-ketocholesterol is degraded faster, and regular cholesterol, which can readily be metabolized by human enzymes, is produced.

Conclusions

A mathematical model based on a system of three differential equations was built using Michaelis-Menten enzyme kinetics in order to quantitatively describe the behaviour of a proposed metabolic pathway for 7-ketocholesterol degradation and atherosclerosis prevention. The model was solved numerically using Runge-Kutta, fourth order approximations, and the results show that, the greater the affinity of the enzymes for 7-ketocholesterol (which means lower Michaelis constant values), the larger will the efficiency of degradation be. When using this differential model, the proposed metabolic pathway, based on *Rhodococcus jostii* metabolism, is predicted to metabolize 7-ketocholesterol way faster than regular human cells' pathways.

References

1. Mathieu JM. *Strategies for the mitigation of oxysterol-induced cytotoxicity*. Texas: Rice University; 2011.
2. Delgado-Cepeda, FJ. *Métodos numéricos para ingeniería*. Mexico: Editorial Digital Tecnológico de Monterrey; 2011.